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Responses of the extrapyramidal and limbic substance P systems to ibogaine and cocaine treatments

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Abstract

Ibogaine is an indolamine found in the West Africa shrub, *Tabernanthe iboga*, and has been proposed for the treatment of addiction to central nervous system (CNS) stimulants such as cocaine and amphetamine. The mechanism of ibogaine action and its suitability as a treatment for drug addiction still remains unclear. Since previous studies demonstrated differential effects of stimulants of abuse (amphetamines) on neuropeptide systems such as substance P, we examined the impact of ibogaine and cocaine on extrapyramidal (striatum and substantia nigra) and limbic (nucleus accumbens and frontal cortex) substance P-like immunoreactivity. Ibogaine and cocaine treatments altered substance P systems by increasing striatal and nigral substance P-like immunoreactivity concentration 12 h after the last drug treatment. However, substance P-like immunoreactivity content was not significantly increased in nucleus accumbens after treatment with either drug. The ibogaine- and cocaine-induced increases in substance P-like immunoreactivity in striatum and substantia nigra were blocked by coadministration of selective dopamine D₁ receptor antagonist (SCH 23390; *R*(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) or dopamine D₂ receptor antagonist (eticlopride; *S*(-)-3-Chloro-5-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride). Most of the responses by substance P systems to ibogaine administration resembled those caused by cocaine, except in cortical tissue where multiple administration of cocaine, but not ibogaine increased substance P-like immunoreactivity. These data suggest that substance P systems may contribute to the effects of ibogaine and cocaine treatment. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The search for new therapeutic agents, which may be useful in the treatment of drug addiction, has been of high priority. Ibogaine (EndabuseTM), the principal alkaloid of the West African shrub *Tabernanthe iboga*, has been studied for the past decade as a potential agent for the treatment of both opioid and stimulant abuse. Anecdotal reports in humans suggest that ibogaine treatment interrupts the physiological and psychological aspects of the withdrawal and dependence phenomena associated with several abused substances, (Lotsof, 1985, 1986, 1995; Sisko 1993). However, the neurochemical bases of the pharmacological actions of ibogaine remain unresolved.

Previous studies demonstrated that in laboratory animals ibogaine antagonizes cocaine-induced locomotor stimulation, reduces a preference for cocaine consumption and diminishes cocaine self-administration (Sershen et al., 1992; Cappendijk and Dzoljic, 1993). Since the addicting properties of cocaine are due to the ability of this drug to increase extracellular dopamine content in extrapyramidal and limbic systems (Dworkin and Smith, 1988; Johnson and Fischman, 1989), it is possible that ibogaine also interacts with the dopaminergic system associated with these brain structures. Previous studies have suggested that ibogaine alters dopamine release in the mesolimbic system (Maisonnette et al., 1991; Maisonnette and Glick, 1992; Glick et al., 1993; Harsing et al., 1994; Reid et al., 1996). However, ibogaine has been shown to have weak affinity for the dopamine receptor sites (Deecher et al., 1992; Schneider et al., 1996) or dopamine transporter (Sershen et al., 1992; Broderick et al., 1994). Thus, ibogaine may affect dopamine activity indirectly due to its actions on

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other neurotransmitter pathways. In addition to interactions between ibogaine and dopaminergic pathways, this drug also affects noradrenergic (Gershon and Lang, 1962; Palumbo and Winter, 1992), serotonergic (Mash et al., 1995a; Sershen et al., 1995), cholinergic (Repke and Artis, 1994); nicotinic (Schneider et al., 1996; Badio et al., 1997; Maisonneuve et al., 1997; Fryer and Lukas, 1999), κ -opioid (Sershen et al., 1995, 1996a), σ -opioid (Bowen et al., 1995; Sershen et al., 1996b), glutamate (Popik et al., 1994; Mash et al., 1995b; Chen et al., 1996; Layer et al., 1996; Sershen et al., 1996b), neuroendocrine (Ali et al., 1996) and neurotensin systems (Alburges and Hanson, 1999a). To evaluate the possibility that other neuropeptide systems may be relevant to the action of ibogaine, the responses of substance P systems to treatment with this drug were examined and compared to those caused by cocaine.

Substance P is a neuropeptide believed to act by stimulating dopaminergic neurons in the substantia nigra and is associated with the excitatory striatonigral projection (Davies and Dray, 1976). This substance P neuronal system has been suggested to have a feedback influence on dopamine nigrostriatal pathways (Cheramy et al., 1977; Pernow, 1983). Thus, it is not surprising that drugs that alter dopamine activity also affect the dynamics of the striatonigral substance P pathway. For example, previous studies reported that changes in activity of dopaminergic pathways induced by amphetamines substantially alter substance P systems (Ritter et al., 1984, 1985; Sonsalla et al., 1984, 1986; Hanson et al., 1986a,b). Because of the apparent interaction between this neuropeptide and dopaminergic systems, substance P may contribute to the effects of the stimulants of abuse. In this study, we examined and compared the response of substance P systems to ibogaine and cocaine treatment by evaluating substance P-like immunoreactivity concentrations in several extrapyramidal and limbic regions. In addition, selective dopamine receptor antagonists were administered alone and in combination with ibogaine or cocaine in order to identify if dopamine receptor subtype(s) were involved in the response of substance P striatonigral circuitry to administration of these drugs. The analysis of the substance P-like immunoreactivity content will provide insight into mechanism(s) potentially involved in addiction to stimulants of abuse such as cocaine and will increase understanding of the interactions of substance P with the dopamine pathways in brain regions commonly affected by drugs of abuse. Knowledge of the mechanism of action of ibogaine on substance P systems may also help to identify potential antiaddictive drugs for the treatment of drug dependence.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (180 to 230 g), acquired from Simonson Laboratories, (Gilroy, CA) were housed

and cared for according to NIH guidelines. Animals were kept on a 12-h light/dark cycle with food and water available ad libitum in a temperature-controlled room. The animals were allowed to acclimate for at least 2 weeks before their use. All experiments were carried out according to the guidelines of the University of Utah Institutional Animal Care and Use Committee.

2.2. Drug treatment

The rats received single or multiple intraperitoneal injections of drugs and were sacrificed 12 h after drug treatment. Treatment protocols consisted of: (a) a single administration of ibogaine (20 or 40 mg/kg, i.p.); (b) multiple daily administrations of ibogaine (40 mg/kg/dose), cocaine (30 mg/kg/dose) or saline solution (0.9% w/v NaCl, pH 7.4) for four consecutive days; or c) four daily injections of SCH 23390 (0.5 mg/kg/dose), or eticlopride (0.5 mg/kg/dose), administered 15 min prior to ibogaine (40 mg/kg/dose), cocaine (30 mg/kg/dose) or saline (1 ml/kg). Doses were calculated as free base of the drug and were prepared in saline solution. Following the last dose of drug treatment, animals were decapitated at the times indicated and brains were removed rapidly and placed on ice after the frontal cortices were removed. The remainder of the brain tissue was frozen immediately on dry ice and the areas of interest including striatum, nucleus accumbens and substantia nigra were dissected from 1-mm thick frozen coronal slices, using a microdissection knife and subsequently stored at -80°C until assayed for substance P-like immunoreactivity.

SCH 23390 hydrochloride (*R*(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and eticlopride hydrochloride (*S*(-)-3-Chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride) were acquired from Research Biochemicals (Natick, MA). Ibogaine hydrochloride and cocaine hydrochloride were generously supplied by the National Institute on Drug Abuse.

2.3. Determination of substance P-like immunoreactivity

Tissue samples were homogenized in 0.01 N HCl. The resulting homogenate was placed in boiling water for 10 min to inactivate peptidases. Homogenates were centrifuged at $\sim 12,000$ rpm for 30 min. Following centrifugation, the supernatant was removed. An aliquot of the supernatant was used to determine the total protein for each tissue with the method of Bradford (1976) and the remainder of the supernatant was lyophilized overnight and stored at -80°C until the radioimmunoassay was performed. The concentrations of substance P-like immunoreactivity were determined with a modified solid-phase radioimmunoassay technique described for neurotensin analysis (Maidment et al., 1991), which reliably detected 0.25 pg of SP. Lyophilized samples were reconsti-

tuted in a phosphate-buffered saline (pH 7.4) containing 0.1% (w/v) of gelatin and 0.1% (v/v) of Triton X-100. Nunc-Immunoplates (ISC BioExpress, Kaysville, UT) were incubated overnight at 4°C with 50 μ l of protein G solution (50 ng/100 μ l in 0.1 M sodium bicarbonate; pH 9.0). After washing the wells three times with wash buffer (0.15 M K_2HPO_4 , 0.02 M NaH_2PO_4 , 0.2 mM ascorbic acid, 0.2% (v/v) Tween 20 and 0.1% (w/v) sodium azide; pH 7.5), 50 μ l of a highly selective antiserum for substance P, previously diluted to 1:200,000 in assay buffer (same as wash buffer containing 0.1% (w/v) gelatin), was added to each well and incubated for 4 h at room temperature, to allow the attachment of the antibody to the protein G-coated surface. After repeating the washing procedure, 25 μ l of samples and standards were incubated overnight at room temperature. The following day, 25 μ l of the labeled peptide (125 I-substance P), diluted with assay buffer to approximately 5000 cpm per 25 μ l, were added to the wells and incubated for 2 h at room temperature. After incubation, wells were washed, separated, placed in 12 $\times$ 75 mm polypropylene tubes and counted in a four-channel Micromedic 4/200 plus gamma counter (Micromedic Systems, Huntsville, AL). The total and nonspecific binding were defined by adding 25 μ l of the labeled peptide to protein G-untreated and -treated wells, respectively. Quantities of substance P-like immunoreactivity were determined by comparing bound to free 125 I-substance P in each sample to a standard curve. The assay allowed reliable detection of 0.25 pg of substance P. The substance P antiserum employed in this study is described elsewhere (Nilsson et al., 1975). This substance P antibody displayed less than 2% cross-reactivity with eledoisin and physalemin and less than 4% cross-reactivity with neurokinin A. However, substance P fragments greater than the C-terminal pentapeptide did cross-react with the antiserum. Thus, it is possible that this analytical technique detects not only intact substance P neuropeptide but also some C-terminal substance P metabolites. Consequently, our measurements are referred to as picograms per milligram of protein of substance P-like immunoreactivity.

2.4. Statistical analysis

Results were expressed as percentages of their respective controls in order to facilitate comparisons between groups (mean values $\pm$ S.E.M.). Control values for 1, 12 and 24 h sacrifice time-course in Fig. 3, were not statistically different and were pooled. The control values (pg of substance P-like immunoreactivity/mg of protein) for each experiment are indicated in the corresponding figure legend. Differences between means were analyzed using one-way analysis of variance with Fisher-Protected Least Significant Difference (PLSD). Differences were considered significant when the probability that they were zero was less than 5%.

3. Results

3.1. Response of the substance P systems to multiple administrations of ibogaine or cocaine

To assess the effect of ibogaine or cocaine on substance P systems, animals were injected as described in Section 2 (treatment protocol, b). This ibogaine dosing regimen was selected because of previous studies which revealed that it attenuates cocaine-induced behavioral response and changes in extracellular content of dopamine and its metabolites (Maisonneuve et al., 1991, 1992; Serhsen et al., 1992; Broderick et al., 1994). The selection of the cocaine dose was also based on previous studies which indicated that 30 mg/kg/dose, i.p., cause significant changes in other extrapyramidal and limbic neuropeptide systems (Hanson et al., 1989; Alburges and Hanson, 1998, 1999a,b). The tissue content of substance P-like immunoreactivity was measured at 12 h after the last drug treatment. The data in Fig. 1 demonstrate that in striatal, nigral, and cortical tissues the concentrations of substance P-like immunoreactivity were significantly higher than controls 12 h after cocaine treatments (130%, 152% and 213% of

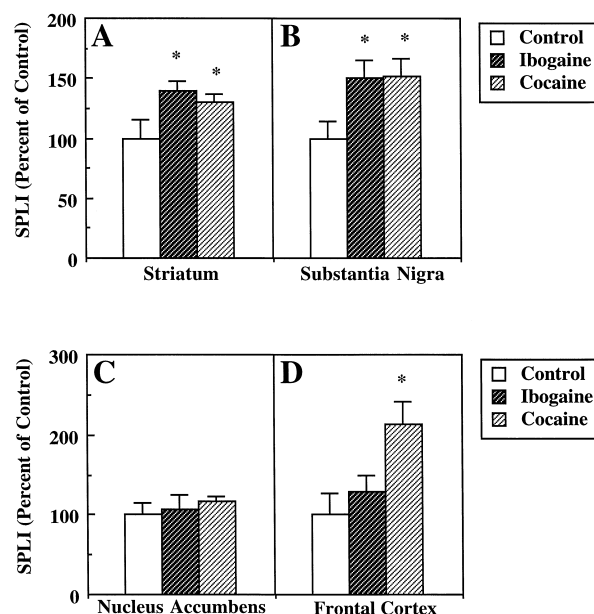


Fig. 1. Effects of multiple administrations of ibogaine or cocaine on substance P-like immunoreactivity concentration in striatum (A), substantia nigra (B), nucleus accumbens (C), and frontal cortex (D). Treatment consisted of one daily injection of ibogaine (40 mg/kg/dose, i.p.), cocaine (30 mg/kg/dose, i.p.), or saline for four consecutive days. Animals were sacrificed 12 h following the last drug administration. The results are presented as percent of control and represent mean values $\pm$ S.E.M. ($n = 6$ control and 9 drug-treated animals per group). The actual control $\pm$ S.E.M. values for substance P-like immunoreactivity contents, as pg/mg of protein, were: striatum, 1500 ± 221 ; substantia nigra, 5099 ± 716 ; nucleus accumbens, 1228 ± 168 ; and frontal cortex, 509 ± 136 . * $P < 0.05$ vs. control group.

control, respectively). While the ibogaine treatment induced significant elevation in substance P-like immunoreactivity concentrations in striatum and substantia nigra (139% and 150% of control, respectively), no alterations of the cortical substance P-like immunoreactivity content occurred (Fig. 1D). Additionally, the substance P-like immunoreactivity content was not altered in nucleus accumbens after either drug treatments (Fig. 1C).

3.2. Dose-effect response of substance P systems to multiple administrations of ibogaine

Animals were injected with varying doses of ibogaine (10, 20 and 40 mg/kg/doses, i.p.) or saline, daily for four consecutive days. Twelve hours after the drug treatments, substance P-like immunoreactivity concentration was significantly increased in striatum and substantia nigra of animals that received 40, but not 10, mg/kg/dose (Fig. 2). Animals which were dosed with 20 mg/kg only had substantially elevated substance P-like immunoreactivity content in the striatum (Fig. 2A, 204% of control). The effects of multiple administrations of different ibogaine doses on substance P-like immunoreactivity content in the limbic system (nucleus accumbens and frontal cortex) were also examined. No significant changes in substance P-like immunoreactivity concentrations of either structure were observed at any of the ibogaine doses administered (data not shown).

3.3. Recovery of the substance P systems from multiple doses of ibogaine

The recovery of striatal and nigral substance P systems after four daily injections of ibogaine (40 mg/kg/dose) was determined by measuring substance P-like immunoreactivity concentrations at 1, 12 and 24 h following the last administration of drug. The striatal content of sub-

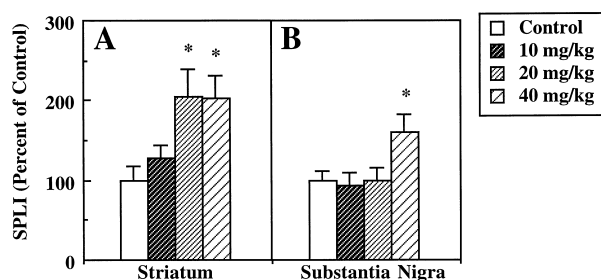


Fig. 2. Effects of multiple administrations of various doses of ibogaine on substance P-like immunoreactivity content in striatum (A) and substantia nigra (B). Animals were given one daily injection of ibogaine (10, 20 or 40 mg/kg/dose, i.p.) or saline for four consecutive days, and sacrificed 12 h after last injection. The results are expressed as percentages of control and represent mean values $\pm$ S.E.M. ($n = 6$ control and 7 drug-treated animals per group). The average control $\pm$ S.E.M. values for substance P-like immunoreactivity concentrations expressed as pg/mg of protein were: striatum, 933 ± 159 and substantia nigra, 6479 ± 769 . * $P < 0.05$ vs. control.

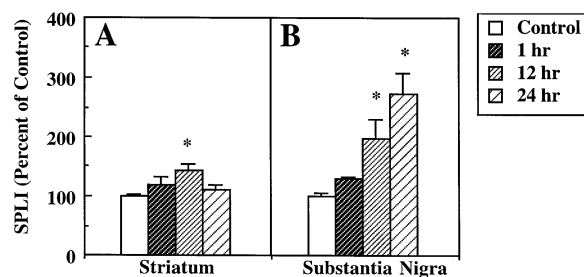


Fig. 3. Temporal response of substance P systems in striatum (A) and substantia nigra (B) to multiple injections of ibogaine. Animals were treated with ibogaine (40 mg/kg/dose, i.p., daily for 4 days) or saline, and sacrificed 1, 12 and 24 h following the last treatment. Control values from 1, 12 and 24 h sacrifice time-course, were not statistically different and were pooled as the control group. The results are presented as percentages of control and represent mean values $\pm$ S.E.M. ($n = 12$ control and 9 drug-treated animals per group). The average control $\pm$ S.E.M. values for substance P-like immunoreactivity contents expressed as pg/mg of protein were: striatum, 1648 ± 72 and substantia nigra, 6833 ± 346 . * $P < 0.05$ vs. control.

stance P-like immunoreactivity was significantly increased to 143% of the control 12 h after treatment (Fig. 3A). The substance P-like immunoreactivity concentration was no longer different from control by 24 h following the ibogaine administration. In the substantia nigra (Fig. 3B), substance P-like immunoreactivity concentrations were significantly elevated at both 12 and 24 h after ibogaine injection (198% and 274% of control, respectively). Results from this experiment indicated that substance P-like immunoreactivity contents in striatum and substantia nigra were comparably changed 12 h following the administration of multiple doses of 40 mg/kg of ibogaine; therefore, this dosing paradigm was used for other experiments in the study.

3.4. Response of substance P systems to a single dose of ibogaine

In order to evaluate the acute effects of ibogaine on substance P systems, rats were injected with a single dose

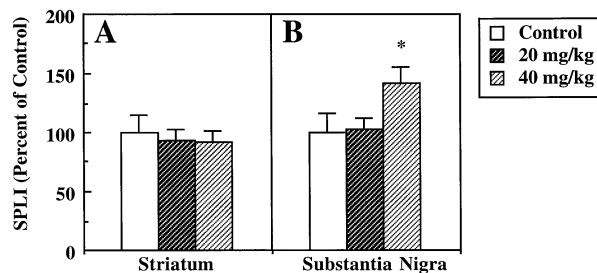


Fig. 4. Effects of a single dose of ibogaine on striatal (A) and nigral (B) substance P-like immunoreactivity content. Animals were administered one injection of ibogaine (20 or 40 mg/kg, i.p.) or saline and sacrificed 12 h after treatment. Results are presented as mean values $\pm$ S.E.M. expressed as percentages of control ($n = 6$ control and 8 drug-treated animals per group). The control values for substance P-like immunoreactivity concentrations (pg/mg of protein) were: striatum, 1895 ± 286 and substantia nigra, 6066 ± 1023 . * $P < 0.05$ vs. control.

of 20 or 40 mg/kg of ibogaine and sacrificed 12 h after drug treatment (Fig. 4). The nigral concentration of substance P-like immunoreactivity was significantly elevated to 142% of the control in the group of animals which received a 40 mg/kg dose (Fig. 4B). A single dose of ibogaine did not cause any significant changes in substance P-like immunoreactivity contents in striatal (Fig. 4A) or limbic regions after any of the doses examined (data not shown).

3.5. Effects of dopamine D_1 and D_2 receptor blockade on ibogaine- and cocaine-induced changes in substance P systems

The role of dopamine receptor subtypes in the ibogaine-induced changes in striatonigral substance P systems was next evaluated and compared with the response to a similar cocaine treatment. Rats were treated as described in Materials and Methods (treatment protocol, c). The data in Fig. 5 demonstrate that ibogaine and cocaine treatment alone significantly elevated the concentrations of substance P-like immunoreactivity in striatum and in sub-

stantia nigra to 137% and 135% (Fig. 5A), and to 176% and 184% (Fig. 5B) of the control, respectively. Pretreatment with either antagonist completely prevented both the ibogaine- and cocaine-induced increases in substance P-like immunoreactivity concentration in striatum and substantia nigra (Fig. 5A and B). The dopamine receptor antagonists (SCH 23390 and eticlopride) by themselves did not significantly affect substance P-like immunoreactivity contents in any of the tissues analyzed.

4. Discussion

In the present study the effects of ibogaine on substance P extrapyramidal and limbic systems were examined and compared to those of cocaine. An important finding of this study was that multiple administration of ibogaine significantly increased the substance P-like immunoreactivity levels in the striatum and substantia nigra, but did not alter substance P-like immunoreactivity levels in the nucleus accumbens or the frontal cortex (Fig. 1). The effects of ibogaine on these extrapyramidal substance P systems were dose-dependent (Fig. 2). A single injection of ibogaine only altered substance P-like immunoreactivity levels in the substantia nigra with the 40 mg/kg dose, but did not affect striatal substance P-like immunoreactivity content even after the high dose. Interestingly, the ibogaine-induced changes in substance P-like immunoreactivity levels appeared to occur maximally at 12–24 h after drug administration with recovery of the effect occurring faster in the striatum (Fig. 3). At this time it is not apparent why the patterns of response to ibogaine by the striatal and nigral substance P systems appear to be somewhat different relative to their sensitivity and temporal response to ibogaine.

In comparison, cocaine administration also increased substance P-like immunoreactivity content in the extrapyramidal structures, but unlike ibogaine, cocaine treatment increased substance P-like immunoreactivity levels in the frontal cortex but not in the accumbens (Fig. 1). The fact that ibogaine appears to have little influence on limbic substance P systems is consistent with our previous observation with other neuropeptides. For example, ibogaine has profound effects on extrapyramidal neurotensin systems without significantly altering limbic neurotensin pathways (Alburges and Hanson, 1999a). Also like substance P pathways, both the limbic and extrapyramidal neurotensin projections are significantly altered by cocaine treatment (Alburges and Hanson, 1999a). Because limbic systems are believed to be critical to the addicting properties of drugs of abuse like cocaine (Wise and Bozarth, 1982; Goeders and Smith, 1983), it may be that ibogaine's apparent preferential actions on extrapyramidal systems and its lack of effect on limbic pathways contribute to its value as a treatment of drugs of abuse such as cocaine. This possibility requires further investigation.

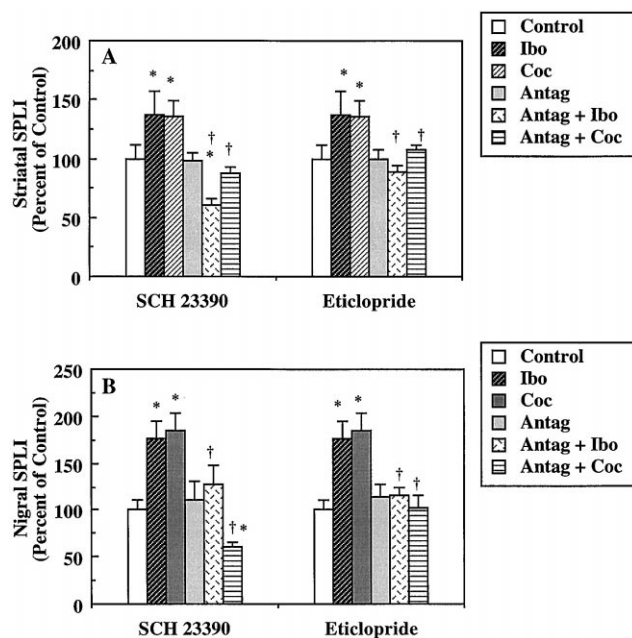


Fig. 5. Effects of selective dopamine receptor antagonists on ibogaine- and cocaine-induced changes in striatal (A) and nigral (B) substance P-like immunoreactivity content. Animals were given a dose of ibogaine (Ibo; 40 mg/kg/dose, i.p.), cocaine (Coc; 30 mg/kg/dose, i.p.) or saline (control), daily for four days alone or 15 min after administration of SCH 23390 (dopamine D_1 receptor antagonist; 0.5 mg/kg/dose, i.p.) or eticlopride (dopamine D_2 receptor antagonist; 0.5 mg/kg/dose, i.p.). Animals were sacrificed 12 h following the last treatment. Values represent the means $\pm$ S.E.M. expressed as percentages of control ($n=6$ control and 8 drug-treated animals per group). The control values $\pm$ S.E.M. for substance P-like immunoreactivity concentrations (pg/mg of protein) were: striatum, 1485 ± 160 and substantia nigra, 7759 ± 768 . * $P < 0.05$ vs. saline control; † $P < 0.05$ vs. corresponding ibogaine or cocaine group.

In order to identify the mechanism responsible for the ibogaine-induced changes in the extrapyramidal substance P systems, the effects of blocking dopamine D₁ and D₂ receptors were examined. The rationale for selecting this strategy to study the ibogaine effect was based on the close association between the nigral–striatal dopamine projection and the substance P-containing striatal–nigral pathway (Davies and Dray, 1976; Cheramy et al., 1977; Waldmeier et al., 1978; James and Starr, 1979). Previous reports have demonstrated that interruption of the nigral–striatal dopamine pathway causes a decrease in substance P-like immunoreactivity content associated with these extrapyramidal structures (Hong et al., 1978; Hanson et al., 1981a,b; Ritter et al., 1984; Sonsalla et al., 1984). Of particular relevance to the present study was the observation by Sonsalla et al. (1986) that blockade of dopamine receptors prevented the increases in striatal and nigral substance P-like immunoreactivity caused by methamphetamine treatment. Interestingly, we observed that pretreatment with selective dopamine D₁ and D₂ receptor antagonists similarly prevented the ibogaine-induced increases in striatal and nigral substance P-like immunoreactivity levels. These findings suggest that by some mechanism ibogaine causes the release of dopamine which in turn alters the activity of the extrapyramidal substance P systems. Consistent with this observation are previous reports that associated ibogaine treatment with changes in dopamine activity. For example: (1) Reid et al. (1996) demonstrated that ibogaine administration induces a biphasic, dose-dependent effect on dopaminergic activity with lower doses inhibiting and higher doses stimulating dopamine release; (2) when directly perfused into the striatum, ibogaine augments dopamine efflux from the mouse striatum (Harsing et al., 1994; Sershen et al., 1996a, 1997); (3) ibogaine blocks morphine-induced increases in striatal dopamine, but augments cocaine- and amphetamine-induced increases in extracellular dopamine (Maisonneuve et al., 1991, 1992; Maisonneuve and Glick, 1992); (4) ibogaine modulates dopamine release mediated by κ -opioid and 5-HT receptors (Sershen et al., 1995, 1996a, 1997; Reid et al., 1996). Although as cited, many studies have associated ibogaine treatment with changes in dopamine activity, the mechanism for this effect is not clear. Some possible targets for ibogaine, which may account for the dopamine impact of this drug, include interaction with: (1) σ -opioid receptors (Bowen et al., 1995; Sershen et al., 1996b); (2) nicotinic receptors (Badio et al., 1997; Maisonneuve et al., 1997); and (3) NMDA receptors (Popik et al., 1994; Mash et al., 1995a,b; Layer et al., 1996; Sershen et al., 1996b). Regardless of the direct site of action of ibogaine, it is clear from the present study that its actions eventually activate both dopamine D₁ and D₂ receptors, which, in turn, alter the activity of extrapyramidal, but not limbic, substance P systems.

We also tested the possibility that cocaine-induced changes in the extrapyramidal substance P systems were

similarly mediated by dopamine receptors. Although this is the first report that cocaine treatment alters the striatal and nigral levels of the substance P peptide, previous studies demonstrated that methamphetamine, another potent stimulant of abuse, also causes increases in extrapyramidal substance P-like immunoreactivity levels by dopamine mechanisms (Ritter et al., 1984). Based on these earlier observations, we anticipated that selective dopamine D₁ and D₂ receptor blockers would alter the substance P response to cocaine. Like with ibogaine, both SCH 23390 and eticlopride completely prevented the striatal and nigral substance P-like immunoreactivity increases induced by cocaine administration suggesting a similar mechanism of action for both drugs on the extrapyramidal substance P systems.

In summary, these studies demonstrated that multiple daily administration of ibogaine produced time- and dose-dependent increases in substance P-like immunoreactivity concentration in extrapyramidal structures. Similar alterations in substance P-like immunoreactivity content also occurred following multiple injections of cocaine. In addition, cocaine treatment increased substance P-like immunoreactivity levels in the frontal cortex, an effect not observed with ibogaine administration. Both drugs appeared to alter striatal and nigral substance P pathways by activating dopamine D₁ and D₂ receptors. These findings suggest that ibogaine and cocaine have similar impact on extrapyramidal systems, but differ considerably in their ability to influence limbic systems. Perhaps the ability of ibogaine to alter substance P systems in this unique manner influences the rewarding properties of cocaine. This possibility is supported by reports that centrally administered substance P can influence reinforcement related behavior (Huston and Oitzl, 1989; Huston et al., 1993) suggesting substance P is associated with reward systems. This possibility deserves further study and may explain some of the anti-abuse properties of ibogaine.

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References

- Alburges, M.E., Hanson, G.R., 1998. Differential effects of ibogaine and cocaine on neurotensin and dynorphin systems. *NIDA Res. Monogr.* 179, 220.
- Alburges, M.E., Hanson, G.R., 1999a. Differential responses by neu-

- rotensin systems in extrapyramidal and limbic structures to ibogaine and cocaine. *Brain Res.* 818, 96–104.
- Alburges, M.E., Hanson, G.R., 1999b. Ibogaine pretreatment dramatically enhances the dynorphin response to cocaine. *Brain Res.* 847, 139–142.
- Ali, S.F., Newport, G.D., Slikker, W., Rothman, R.B., Baumann, M.H., 1996. Neuroendocrine and neurochemical effects of acute ibogaine administration: a time course evaluation. *Brain Res.* 737, 215–220.
- Badio, B., William, L., Daly, J.W., 1997. Ibogaine: a potent noncompetitive blocker of ganglionic/neuronal nicotinic receptors. *Mol. Pharmacol.* 51, 1–5.
- Bowen, W.D., Vilner, B.J., Williams, W., Bertha, C.M., Kuehne, M.E., Jacobson, A.E., 1995. Ibogaine and its congeners are σ_2 receptor-selective ligands with moderate affinity. *Eur. J. Pharmacol.* 279, R1–R3.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Broderick, P.A., Phelan, F.T., Eng, F., Wechsler, R.T., 1994. Ibogaine modulates cocaine responses which are altered due to environmental habituation: in vivo microvoltammetric and behavioral studies. *Pharmacol. Biochem. Behav.* 49, 711–728.
- Cappendijk, S.L., Dzoljic, M.R., 1993. Inhibitory effects of ibogaine on cocaine self-administration in rats. *Eur. J. Pharmacol.* 214, 261–265.
- Chen, K., Kokate, T.G., Donevan, S.D., Carroll, F.I., Rogawski, M.A., 1996. Ibogaine block of the NMDA receptor: in vitro and in vivo studies. *Neuropharmacology* 35, 423–431.
- Cheramy, A., Nieoullon, A., Michelot, R., Glowinski, J., 1977. Effects of intranigral application of dopamine and substance P on the in vivo release of newly synthesized [3 H]-dopamine in the ipsilateral caudate nucleus of the cat. *Neurosci. Lett.* 48, 105–109.
- Davies, J., Dray, A., 1976. Substance P in the substantia nigra. *Brain Res.* 107, 623–627.
- Decher, D.C., Teitler, M., Soderlund, D.M., Bornmann, W.G., Kuehne, M.E., Glick, S.D., 1992. Mechanisms of action of ibogaine and harmaline congeners based radioligand binding studies. *Brain Res.* 571, 242–247.
- Dworkin, S.I., Smith, J.E., 1988. Neurobehavioral pharmacology of cocaine. *NIDA Res. Monogr.* 88, 185–198.
- Fryer, J.D., Lukas, R.J., 1999. Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. *J. Exp. Pharmacol. Exp. Ther.* 288, 88–92.
- Gershon, S., Lang, W.J., 1962. A psycho-pharmacological study of some indoles alkaloids. *Arch. Int. Pharmacodyn.* 135, 31–56.
- Glick, S.D., Rossman, K., Wang, S., Dong, N., Keller, R.W., 1993. Local effects of ibogaine on extracellular levels of dopamine and its metabolites in nucleus accumbens and striatum: interactions with d-amphetamine. *Brain Res.* 628, 201–208.
- Goeders, N.E., Smith, J.E., 1983. Cortical dopaminergic involvement in cocaine reinforcement. *Science* 221, 773–775.
- Hanson, G.R., Alphas, L., Pradhan, S., Lovenberg, W., 1981a. Response of striatonigral substance P systems to a dopamine receptor agonist and antagonist. *Neuropharmacology* 20, 541–548.
- Hanson, G.R., Alphas, L., Wolf, N., Levine, R., Lovenberg, W., 1981b. Haloperidol-induced reduction of nigral substance P-like immunoreactivity: a probe for the interactions between dopamine and substance P neuronal systems. *J. Pharmacol. Exp. Ther.* 218, 568–574.
- Hanson, G.R., Letter, A., Merchant, K., Gibb, J.W., 1986a. Comparison of responses by striatonigral substance P and neurokinin A systems to methamphetamine treatment. *Peptides* 7, 983–987.
- Hanson, G.R., Ritter, J.K., Schmidt, C.J., Gibb, J.W., 1986b. Response of mesolimbic substance P systems to methamphetamine treatment. *Eur. J. Pharmacol.* 128, 265–268.
- Hanson, G.R., Smiley, P., Johnson, M., Letter, A., Bush, L., Gibb, J.W., 1989. Response by the neurotensin systems of the basal ganglia to cocaine treatment. *Eur. J. Pharmacol.* 160, 23–30.
- Harsing, L.G., Sershen, H., Lajtha, A., 1994. Evidence that ibogaine releases dopamine from the cytoplasmic pool in isolated mouse striatum. *J. Neural Transm.* 96, 215–225.
- Hong, J.S., Yang, H.Y., Costa, E., 1978. Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. *Neuropharmacology* 17, 83–85.
- Huston, J.P., Hasenöhrl, R.U., Boix, F., Gerhardt, P., Schwarting, R.K.W., 1993. Sequence-specific effects of neurokinin substance P on memory, reinforcement, and brain dopamine activity. *Psychopharmacology* 112, 147–162.
- Huston, J.P., Oitzl, M.S., 1989. The relationship between reinforcement and memory: parallels in the rewarding and mnemonic effects of the neuropeptide substance P. *Neurosci. Biobehav. Rev.* 13, 171–180.
- James, T.A., Starr, M.S., 1979. Effects of substance P injected into the substantia nigra. *Br. J. Pharmacol.* 65, 423–429.
- Johnson, C.E., Fischman, M.W., 1989. The pharmacology of cocaine related to its abuse. *Pharmacol. Rev.* 41, 3–52.
- Layer, R.T., Skolnick, P., Bertha, C.M., Bandarage, U.K., Kuehne, M.E., Popik, P., 1996. Structurally modified ibogaine analogs exhibit differing affinities for NMDA receptors. *Eur. J. Pharmacol.* 309, 159–165.
- Lotsof, H.S., 1985. Rapid method for interrupting the narcotic addiction syndrome, US patent 4,499,096.
- Lotsof, H.S., 1986. Rapid method for interrupting the cocaine and amphetamine abuse syndrome, US patent 4,587,243.
- Lotsof, H.S., 1995. Ibogaine in the treatment of chemical dependency disorders: clinical perspectives. *Multidisciplinary Association for Psychedelic Studies* 5, 16–27.
- Maidment, N.T., Siddall, B.J., Rudolph, V.R., Erdelyi, E., Evans, C.J., 1991. Dual determination of extracellular cholecystokinin and neurotensin fragments in rat forebrain: microdialysis combined with a sequential multiple antigen radioimmunoassay. *Neuroscience* 45, 81–93.
- Maisonneuve, I.M., Glick, S.D., 1992. Interactions between ibogaine and cocaine in rats: in vivo microdialysis and motor behavior. *Eur. J. Pharmacol.* 212, 263–266.
- Maisonneuve, I.M., Keller, R.W., Glick, S.D., 1992. Interactions of ibogaine and d-amphetamine: in vivo microdialysis and motor behavior in rats. *Brain Res.* 579, 87–92.
- Maisonneuve, I.M., Mann, G.L., Deibel, C.R., Glick, S.D., 1997. Ibogaine and the dopaminergic response to nicotine. *Psychopharmacology* 129, 249–256.
- Maisonneuve, M.I., Keller, R.W., Glick, S.D., 1991. Interactions between ibogaine, a potential anti-addictive agent, and morphine: an in vivo microdialysis study. *Eur. J. Pharmacol.* 199, 35–42.
- Mash, D.C., Staley, J.K., Baumann, M.H., Rothman, R.B., Hearn, W.L., 1995a. Identification of a primary metabolite of ibogaine that targets serotonin transporters and elevates serotonin. *Life Sci.* 57, 45–50.
- Mash, D.C., Staley, J.K., Pablo, J.P., Holohean, A.M., Hackman, J.C., Davidoff, R.A., 1995b. Properties of ibogaine and its principal metabolite (12-hydroxyibogaine) at the MK-801 binding site of the NMDA receptor complex. *Neurosci. Lett.* 192, 53–56.
- Nilsson, G., Larsson, L.I., Hakanson, R., Brodin, E., Pernow, B., Sundler, F., 1975. Localization of substance P-like immunoreactivity in mouse gut. *Histochemistry* 43, 97–99.
- Palumbo, P.A., Winter, J.C., 1992. Stimulus effects of ibogaine in rats trained with yohimbine, DOM, or LSD. *Pharmacol. Biochem. Behav.* 43, 1221–1226.
- Pernow, B., 1983. Substance P. *Pharmacol. Rev.* 35, 85–141.
- Popik, P., Layer, R.T., Skolnick, P., 1994. The putative anti-addictive drug ibogaine is a competitive inhibitor of [3 H]MK-801 binding to the NMDA receptor complex. *Psychopharmacology* 114, 672–674.
- Reid, M.S., Hsu, K., Souza, K.H., Broderick, P.A., Berger, S.P., 1996. Neuropharmacological characterization of local ibogaine effects on dopamine release. *J. Neural Transm.* 103, 967–985.
- Repke, D.B., Artis, J.T., 1994. Nelson, abbreviated ibogaine congeners. Synthesis and reactions of tropan-3-yl-2-and-3-indoles. Investigation of an unusual isomerization of 2-substituted indoles using computational and spectroscopic techniques. *J. Org. Chem.* 59, 2164.

- Ritter, J.K., Schmidt, C.J., Gibb, J.W., Hanson, G.R., 1984. Increases of substance P-like immunoreactivity within striatal–nigral structures after subacute methamphetamine treatment. *J. Pharmacol. Exp. Ther.* 229, 487–492.
- Ritter, J.K., Schmidt, C.J., Gibb, J.W., Hanson, G.R., 1985. Dopamine-mediated increases in nigral substance P-like immunoreactivity. *Biochem. Pharmacol.* 34, 3161–3166.
- Schneider, A.S., Nagel, J.E., Mah, S.H., 1996. Ibogaine selectively inhibits nicotine receptor-mediated catecholamine release. *Eur. J. Pharmacol.* 317, R1–R2.
- Sershen, H., Hashim, A., Harsing, L.G., Lajtha, A., 1992. Ibogaine antagonizes cocaine-induced locomotor stimulation in mice. *Life Sci.* 50, 1079–1086.
- Sershen, H., Hashim, A., Lajtha, A., 1995. The effect of ibogaine on *k*-opioid- and 5-HT₃-induced changes in stimulation-evoked dopamine release in vitro from striatum of C57BL/6By mice. *Brain Res. Bull.* 36, 587–591.
- Sershen, H., Hashim, A., Lajtha, A., 1996a. Effect of ibogaine on cocaine-induced efflux of [³H]dopamine and [³H]serotonin from mouse striatum. *Pharmacol. Biochem. Behav.* 53, 863–869.
- Sershen, H., Hashim, A., Lajtha, A., 1996b. The effect of ibogaine on sigma- and NMDA-receptor-mediated release of [³H]dopamine. *Brain Res. Bull.* 40, 63–67.
- Sershen, H., Hashim, A., Lajtha, A., 1997. Ibogaine and cocaine abuse: pharmacological interactions at dopamine and serotonin receptors. *Brain Res. Bull.* 42, 161–168.
- Sisko, B., 1993. Interrupting drug dependency with ibogaine: a summary of four case histories. *Multidisciplinary Association for Psychedelic Studies* 4, 15–24.
- Sonsalla, P.K., Gibb, J.W., Hanson, G.R., 1984. Opposite responses in the striato-nigral substance P system to D₁ and D₂ receptor activation. *Eur. J. Pharmacol.* 105, 185–187.
- Sonsalla, P.K., Gibb, J.W., Hanson, G.R., 1986. Nigrostriatal dopamine actions on the D₂ receptors mediate methamphetamine effects on the striatonigral substance P system. *Neuropharmacology* 25, 1221–1230.
- Waldmeier, P.C., Kam, R., Stocklin, K., 1978. Increased dopamine metabolism in rat striatum after infusions of substance P into the substantia nigra. *Brain Res.* 159, 223–227.
- Wise, R.A., Bozarth, M.A., 1982. Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. *Pharmacol. Biochem. Behav.* 17, 239–243.